UNIVERSITI PUTRA MALAYSIA

INDUCTION STRATEGIES FOR INTERFERON-α2B PRODUCTION IN PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

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INDUCTION STRATEGIES FOR INTERFERON-α2B PRODUCTION IN PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

INDUCTION STRATEGIES FOR INTERFERON-α2B PRODUCTION IN PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

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Chair: Assoc. Prof. Mohd Puad Abdullah, PhD

Faculty: Institute of Bioscience

Interferon-α2b (IFN-α2b) is a member of cytokine family with the ability to induce antiproliferative, antiviral, antineoplastic and immunomodulating activities. It is frequently used in the treatments of gastrointestinal tract diseases, various cancers and chronic hepatitis B. Due to its diagnostic and therapeutic potentials the production of IFN-α2b in large quantities is required to meet the demands from research, clinical and industrial applications. This study was carried out to enhance the production of recombinant IFN-α2b in the periplasmic space of *E. coli* using the recombinant *E. coli* Rosetta-gami 2(DE3) harboring the plasmid pET26b-IFN-α2b. In this study, different induction parameters (temperature, IPTG concentration, point of induction and post induction time) were analyzed and optimized during induction for optimal periplasmic IFN-α2b production. Further analysis was done on the effect of pH medium on IFN-α2b production in different fractions.

Preliminary screening performed on four induction parameters shows that only three parameters (temperature, IPTG concentration and point of induction) improved the

IFN-α2b production. These parameters were optimized individually by narrowing the working range as follows: temperature from 16°C to 30°C, IPTG lower than 2 mM, and induction point between early-log phase and mid-log phase on the growth curve of *E. coli*. A response surface methodology (RSM) based on a central composite design was used to optimize the induction parameters. The proposed induction strategy consisted of three optimized parameters: i) induction point at A_{600} of 4, ii) IPTG strength at 0.05 mM, and iii) induction temperature at 25°C. The strategy yielded 1.21 μg/mL of IFN-α2b, which represents 82% of the soluble IFN-α2b that was successfully transferred to the periplasmic space of *E. coli* after 18 h of induction.

A study on the effect of pH on IFN-α2b production in different fractions reveals that the highest yield was obtained at pH 7 with a high buffering capacity (0.2 M). At this pH, a higher IFN-α2b production was obtained in the periplasmic fraction. Whereas, the amount of inclusion bodies formed was reduced. This was evident by the lack of aggregated materials in the cytoplasm of *E. coli* observed under the electron microscope. This study also proved that the suitable buffering capacity would maintain the pH of media during fermentation.

Finally, when the optimized induction parameters with the appropriate media pH was applied, the total IFN-α2b obtained was 1.43 μg/mL, a 86.9-fold increase in productivity compared to the IFN-α2b produced under non-optimal condition (16.26 ng/mL). Thus, proper optimization of fermentation conditions was proved useful to improve the production of periplasmic IFN-α2b in *E. coli*.

Abstrak tesis untuk dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

STRATEGI INDUKSI UNTUK PENGHASILAN INTERFERON-α2B DI DALAM PERIPLASMIK *ESCHERICHIA COLI*

Oleh

SITI NOR ANI AZAMAN

Januari 2011

Pengerusi: Prof. Madya Mohd Puad Abdullah, PhD

Fakulti: Institut Biosains

Interferon-α2b (IFN-α2b) ialah protein yang tergolong dalam kumpulan sitokin yang mempunyai kebolehan untuk menginduksi aktiviti anti-penggandaan, anti-virus, anti-neoplastik dan imunomodulasi. Ia sering digunakan dalam rawatan penyakit seperti penyakit saluran pencernaan, pelbagai jenis kanser dan hepatitis B kronik. Berdasarkan potensinya dalam bidang diagnostik dan terapeutik, pengeluaran IFN-α2b diperlukan bagi memenuhi permintaan dalam sektor penyelidikan, klinikal dan perindustrian. Kajian ini dijalankan untuk meningkatkan penghasilan rekombinan IFN-α2b di dalam ruang periplasmik *E. coli* menggunakan *E. coli* rekombinan Rosetta-gami 2 (DE3) yang mengandungi plasmid pET26b-IFN-α2b. Dalam kajian ini, beberapa parameter induksi (suhu, kepekatan IPTG, masa induksi dan tempoh induksi) dianalisis dan dioptimumkan untuk penghasilan IFN-α2b yang optimum. Analisis selanjutnya telah dilakukan ke atas kesan pH media ke atas penghasilan IFN-α2b di dalam bahagian yang berlainan.

Penyaringan awal ke atas empat parameter induksi menunjukkan hanya tiga parameter induksi (suhu induksi, kepekatan IPTG dan masa induksi) dapat meningkatkan penghasilan IFN-α2b. Ketiga-tiga parameter ini dioptimumkan secara individu dengan mengecilkan julat kerja seperti berikut: suhu induksi daripada 16°C hingga 30°C, kepekatan IPTG kurang daripada 2 mM, dan masa induksi antara fasa awal-log hingga fasa pertengahan-log pada lengkung pertumbuhan *E. coli*. Kaedah respon permukaan (RSM) berdasarkan reka bentuk komposit pusat telah digunakan untuk mengoptimumkan parameter induksi. Strategi induksi yang dicadangkan mengandungi tiga parameter optimum: i) masa induksi pada A_{600} 4, ii) kepekatan IPTG pada 0.05 mM, dan iii) suhu induksi pada 25°C. Strategi induksi yang optimum telah menghasilkan 1.21 μg/mL IFN-α2b, yang mewakili 82% daripada IFN-α2b larut yang berjaya dipindahkan ke ruang periplasmik *E. coli* selepas 18 jam induksi.

Kajian mengenai kesan pH ke atas penghasilan IFN-α2b di dalam pecahan yang berlainan membuktikan bahawa hasil yang paling tinggi telah diperoleh pada pH 7 dengan menggunakan kapasiti penimbal yang tinggi (0.2 M). Pada pH ini, penghasilan IFN-α2b yang tinggi telah diperolehi daripada fraksi periplasmik dengan pengurangan jumlah pembentukan jasad rangkuman. Ini dibuktikan dengan berkurangnya molekul aggregat di ruang sitoplasmik *E. coli* yang diperhatikan di bawah mikroskop elektron. Kajian ini juga membuktikan penggunaan kapasiti penimbal yang sesuai dapat mengawal pH media semasa fermentasi.

Akhirnya, apabila parameter induksi yang telah dioptimakan digunapakai dengan pH media yang sesuai, jumlah keseluruhan IFN-α2b yang diperolehi ialah 1.43 μg/mL,

86.9 kali ganda peningkatan dari segi produktiviti berbanding IFN-α2b yang dihasilkan pada keadaan tidak optimum (16.26 ng/mL). Maka, pengoptimuman keadaan fermentasi yang terbaik telah terbukti berguna bagi meningkatkan penghasilan periplasmik IFN-α2b dalam *E. coli*.

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I certify that a Thesis Examination Committee has met on **14 January 2011** to conduct the final examination of Siti Nor Ani Azaman on her thesis entitled "**Induction Strategies for Interferon-α2b Production in the Periplasmic Space of *Escherichia Coli***" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)] 15 March 1998. The Committee recommends that the student be awarded the degree of Master Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SITI NOR ANI AZAMAN

Date: 14 January 2011

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